DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC (PBPK/PD) MINIPIG MODEL FOR SIMULATION OF LOW LEVEL MULTIPLE ROUTE CW AGENT EXPOSURE

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ABSTRACT

A physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model was developed to simulate the concentration and effects of low-level chemical warfare agents (CWA) in the Göttingen minipig. The model code was written to account for absorption of CWAs from multiple sites (respiratory tract – lower and upper, dermal, ocular) after vapor exposure. Literature references to minipig physiology were used for the majority of organ volumes and blood flows, while some parameter values were scaled from other species. Unique features of this PBPK/PD model structure were physiological compartments for the eyes, as a source of external CWA absorption and internally as a site of ChE binding, and skin as a dermal absorption pathway. One initial pharmacodynamic endpoint developed in this model was CWA inhibition of cholinesterases (AChE, BChE), with a particular focus on the dynamics of miosis. Preliminary assumptions were that pupil constriction (miosis) from external, systemic or combined delivery of CWA, could be predicted based on AChE inhibition at the ocular muscules. The PBPK/PD model was used to simulate AChE inhibition after inhalation of CWA and to predict potential pharmacodynamic effects at different tissue target sites. This preliminary model will provide a quantitative tool to predict the physiological consequences of low level, non-lethal exposure after CWA exposure.

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INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models have become useful analytical tools to interpret pharmacokinetic data. These models are mathematical constructs that allow the coordination of species specific physiology, chemical specific characteristics and the experimental protocol for the chemical and exposures of concern. The power of PBPK models lie in aiding the ability of scientists and decision makers to simulate the time-course concentration of chemicals in experimental animals and humans, to determine better estimates of actual chemical doses. Due to the physiologically based nature of these models, simulations of experimental data can be performed by one exposure route, to validate the PBPK model, and then this model can be used to simulate and predict the kinetics and pharmacodynamics, in the human by one or multiple exposure routes. This provides decision makers with a fairly rapid method of comparing results from *in vitro* and *in vivo* laboratory studies, to potentially real world exposure scenarios.

There are generally three critical components to PBPK models: 1) Species specific physiological parameters, 2) Chemical specific parameters, and 3) Specific experimental protocol. Species-specific physiological parameters are the organ weights and blood flow rates for the defined compartments in the PBPK model. These values are most often available in the published literature and when lacking, are derived from the closest like species. In this particular study, the experimental studies simulated were conducted with the Göttingen minipig as the animal model. The minipig has become a very useful experimental model due to its anatomical and physiological similarities to humans (Svendson 1998). The size of the young mature animal lends itself to many experimental techniques that would not be possible with smaller species, yet it is still small enough to not require large exposure facilities (Koch et al. 2001). This is the first reported effort to develop a PBPK model for the Göttingen minipig. This required surveying the literature for anatomical and physiological values specific for this strain of minipig. Chemical specific parameters that are unique for each chemical are the tissue solubility (partition coefficient), metabolism of the parent compound, and plasma and tissue binding characteristics. Tissue solubility is most often measured experimentally by the vial equilibration method (Gargas et al. 1989).

METHODS

The basic structure of the PBPK model used to describe sarin pharmacokinetics and pharmacodynamics (PD) was based on the PBPK/PD model for diisopropylfluorophosphate (DFP) (Gearhart et al. 1990). Tissue compartments (Figure 1) that were added to the previous model structure were the eye and the skin, where previously these compartments were lumped together in the rapidly perfused or slowly perfused tissues. The eye was added to provide the means of predicting miosis during CW agent exposure, from both the systemic absorption of chemicals, but more importantly, the amount of chemical absorbed directly to the eye structures via the ocular surface via absorption and diffusion. The skin was added primarily to provide an exposure route for those CW agents that have a significant dermal absorption potential.

PBPK parameters for individual organ weights were obtained from the web page of the Ellegaard Co. (http://www.minipigs.dk/), which is a provider of Göttingen minipigs. Blood flows for most organs were obtained from the Armstrong et al. (1987). The partition coefficients were based on the values used for DFP (Gearhart et al. 1990) and soman (Langenberg et al. 1997), as well as the cholinesterase and sarinase values for tissue and blood (Figure 2).

RESULTS

The simulaton of RBC AChE inhibition in minipigs exposed by inhalation to 0.17 mg/m^3 is shown in figure 3. Initial simulations of AChE inhibition which assumed all of the inhaled agent reached the lung and was absorbed directly into the systemic circulation provide a significant over prediction of AChE inhibition. To correct for this over prediction, the model structure was changed to add deposition of Sarin into the upper airways (URT) during inhalation, thus preventing differing fractions of the inhaled concentration from reaching the respiratory surfaces and allowing gas exchange and absorption. Assuming 50% absorption of sarin in the URT provided the closest prediction of the data at 80% measured AChE inhibition (Figure 3). At these lower sarin levels, the biological varitation in the experimental data is greater and the RBC AChE shows an immediate rebound to near control levels. Figure 3 shows the effects of successive increases in the deposition of sarin on the inhibition of AChE. As shown in figures 4 and 5, the analysis with the PBPK/PD model of the three different endpoints provides the best estimate of the actual URT scrubbing of sarin.

Figure 4 presents simulations of the data of Jakabowski et al. (2003) with 0%, 50%, 75% and 90% URT deposition of sarin and it's effect on predicting data from regenerated sarin bound on RBCs. Clearly, this graph shows that for the same exposure concentration and duration as the data in figure 3, 90% URT sarin loss provides the best estimate of the data from this assay method. The regeneration method of Jakabowski represents a closer measure of the actual delivered dose than the AChE inhibition, which is an actual biological response.

Figure 5 shows the data of Hulet et al (2002), again simulated with 0, 50 or 75% URT deposition of sarin and it's effect on the prediction of pupil area. This figure does not show the 90% URT effects, but clearly exhibits the fairly good overall prediction of miosis for the first 45-50 minutes of sarin exposure, at which point the data shows a precipitous decrease in pupil diameter out to and beyond the end of exposure. The simulations of these data did not assume any direct ocular absorption to the outer eye surface and diffusion to the retinal muscles. This exercise shows that the early time-course of miosis is predominately due to sustemic delivery of sarin to the retinal muscles via the general circulation. The hypothesis from this data set indicates there is a variable period of time, dependent on concentration and exposure duration, which dictates the onset of miosis from direct CW agent contacting the eye.

CONCLUSIONS

A preliminary PBPK/PD model was developed for the Göttingen minipig, which advanced the efforts of an earlier model developed to simulate the cholinesterase inhibition of DFP. This new model was equipped with specific compartments for the eye and skin, to allow these two organs to be simulated as both a route of entry (skin) of CW agents and a target site (eye – miosos). This new model was successfully used to simulate the effects of sarin inhalation on AChE inhibition, measurement of regenerated RBC-sarin and the onset of miosis resulting from systemic delivery of agent in the blood supply to the retina of the eye. The model was not exercised to predict the absorption of sarin at the ocular surface, yet, as parameters for this process are still being developed. It is expected that once the ocular absorption rate for sarin and the diffusion of agent to the retinal muscles is parameterized in the model, it will be possible to separate the effects of systemically delivered agent on miosis versus that which occurs from direct air exposure to the eye surface.

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Figure 1. PBPK/PD Model Schematic of Sarin in Gottingen Minipig

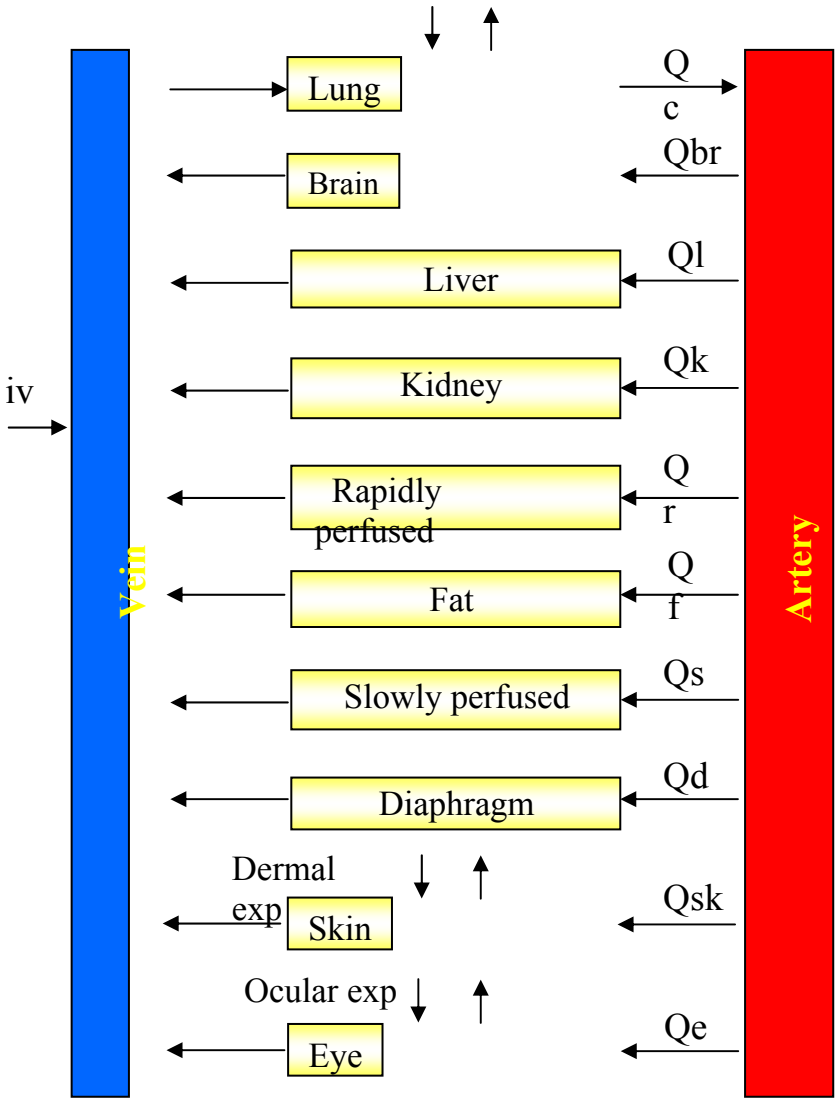

Figure 2. Model of acetylcholinesterase inhibition, aging, regeneration, synthesis and degredation.

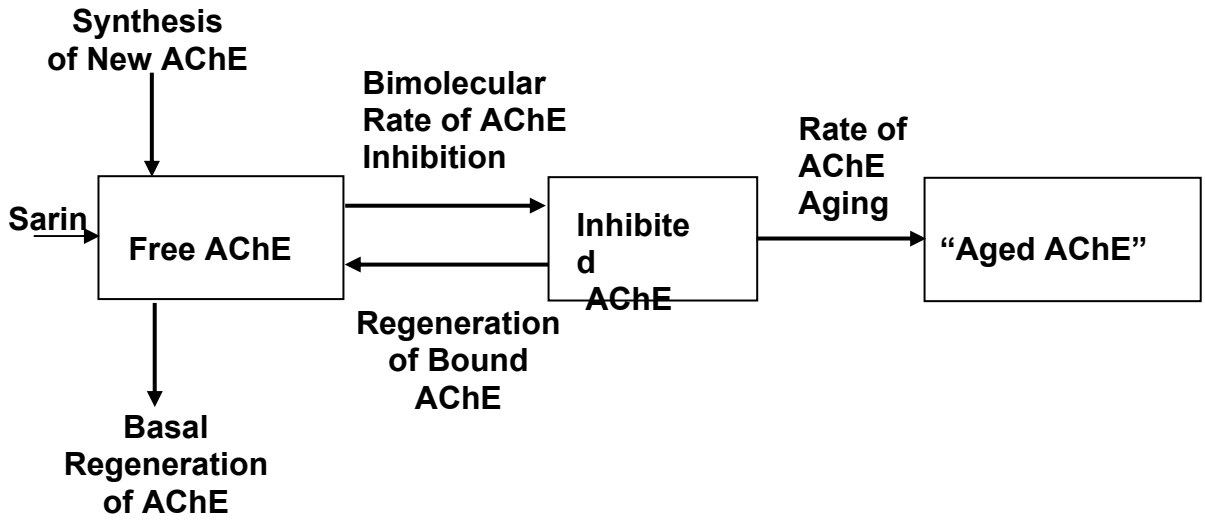

Figure 3. PBPK/PD simulation (solid lines) of the inhibition of red blood cell acetylcholinesterase (AchE) in a minipig after a ten minute inhalation exposure to 0.17 mg/m^3 sarin. (Data from Hulet et al. 2002). The simulation lines represent AChE inhibition after deposition of Sarin in upper respiratory tract (URT) at 50%, 75% or 90% loss.

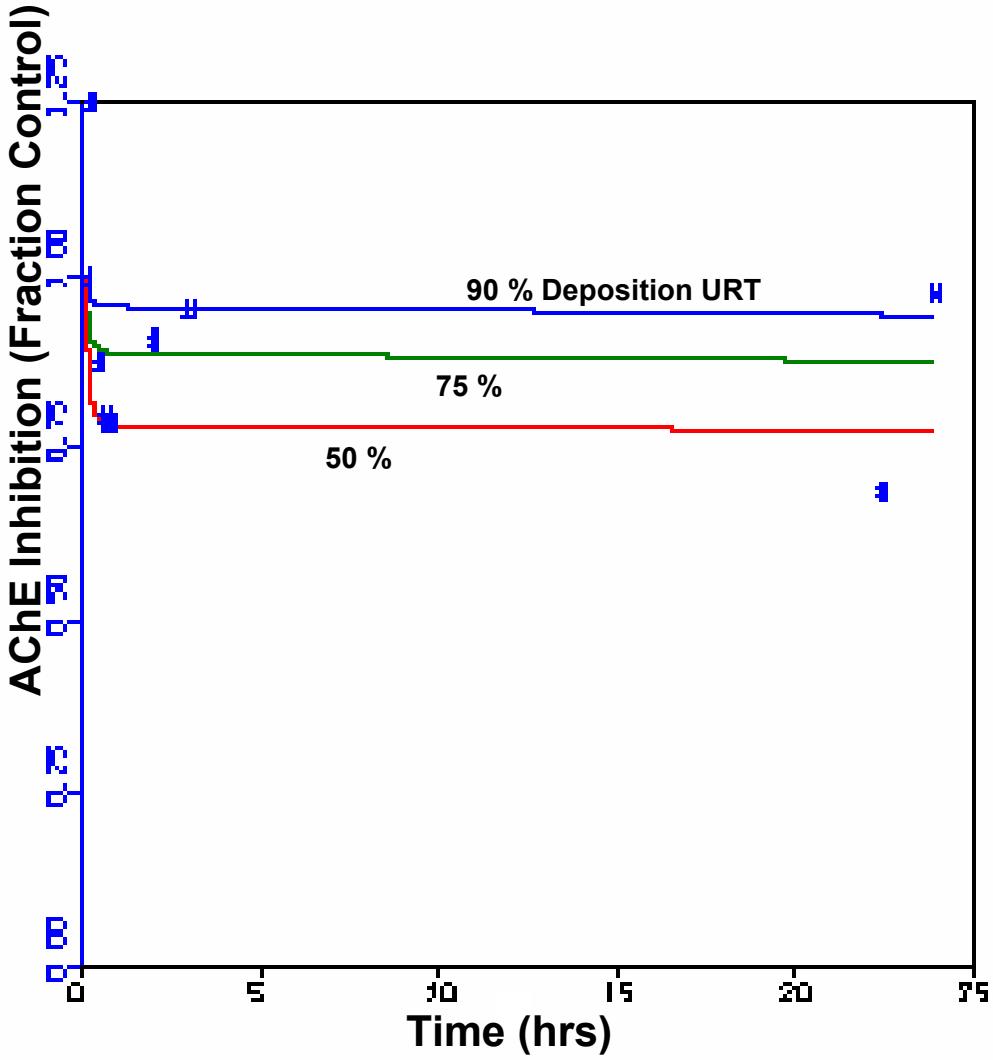

Figure 4. PBPK/PD simulation (solid lines) of regenerated sarin bound to red blood cell acetylcholinesterase in a minipig after a ten minute inhalation exposure to 0.17 mg/m^3 sarin. (Data from Jakabowski et al. 2003). The simulation lines represent regenerated RBC Sarin after upper respiratory tract (URT) deposition at 50%, 75% or 90% loss.

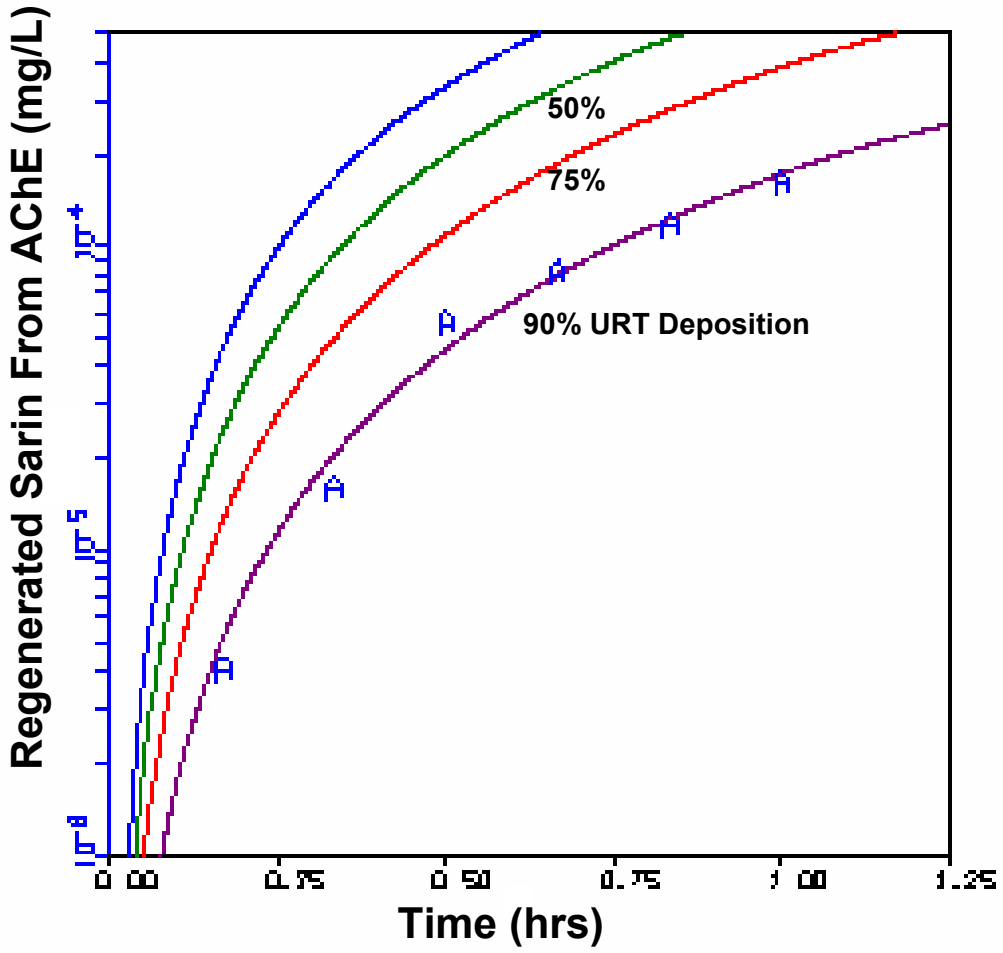

Figure 5. PBPK/PD simulation (solid lines) of pupil area in a minipig during and after a 60 minute inhalation exposure to 0.047 mg/m^3 sarin. (Data from Hulet et al. 2002). The simulation lines represent miosis after deposition of Sarin in upper respiratory tract (URT) at 50%, 75% or 90% loss.